

IN THE SPECIFICATION:

On page 1, after the title, please add the following paragraph:

--Cross-Reference to Related Application

This application is a divisional application of U.S. Patent Application Serial No. 09/836,134, filed April 16, 2001, which is a divisional application of Serial No. 09/077,055, filed August 3, 1998, entitled "Epothilons C and D, Preparation and Compositions", which was filed under 35 U.S.C. § 371 from PCT/EP96/05080, filed November 18, 1996. --

Please rewrite on page 12, the paragraph beginning at line 3 as follows:

100 mg (0.203 mmol) of epothilon A are dissolved in 3 ml of pyridine, 50 μ l (0.686 mmol) of thionyl chloride are added and the reaction mixture is stirred at room temperature for 15 minutes. 1M Phosphate buffer pH 7 is then added and the aqueous phase is extracted four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The crude product is purified and the four stereoisomers 3a-d are separated by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Please rewrite on page 17, the paragraphs beginning at lines 9 and 12 as follows:

5 mg (0.009 mmol) of 3,7-di-O-acetyl-epothilon A are dissolved in 1 ml of methanol, 150 μ l of an ammoniacal methanol solution (2 mmol NH_3 /ml methanol) are added and the reaction mixture is stirred overnight at 50°C. For separation, the solvent is removed *in vacuo*. The raw product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Please rewrite on page 18, the paragraphs beginning at lines 3 and 6 as follows:

20 mg (0.041 mmol) of epothilon A are dissolved in 0.5 ml of methanol, 0.5 ml of 1N sodium hydroxide solution is added and the reaction mixture is stirred at room temperature for 5 minutes.

The reaction mixture is worked up by adding 1M phosphate buffer pH 7 and extracting the aqueous phase four times with ethyl acetate. The combined organic phases are washed with saturated sodium chloride solution, dried over sodium sulphate and freed of solvent. The raw product is purified by preparative layer chromatography (eluant : dichloromethane/methanol, 85:15).

Please rewrite on page 19, the paragraph beginning at line 3 as follows:

5 mg (0.009 mmol) of 7-O-acetyl-epothilon A are dissolved in 1 ml of methanol, 200 μ l of an ammoniacal methanol solution (2 mmol NH_3 /ml methanol) are added and the reaction mixture is stirred at 50°C for two days. For separation, the solvent is removed *in vacuo*. The raw product is purified by preparative layer chromatography (eluant: toluene/methanol, 90:10).

Please rewrite on page 20, the paragraphs beginning at lines 4 and 17 as follows:

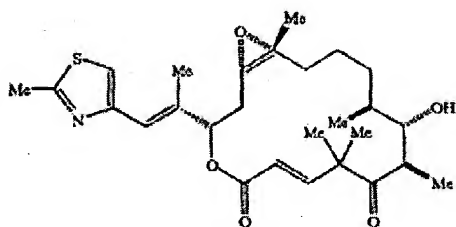
48 mg of epothilonic A acid 7 are dissolved in 6 ml of abs. THF and, with stirring, 40 μ l of triethylamine and 16 μ l of 2,4,6-trichlorobenzoyl chloride are added. After 15 minutes, the precipitate is removed by filtration and the filtrate is added dropwise, within a period of 15 minutes, with rapid stirring, to a boiling solution of 20 mg of 4-dimethylaminopyridine in 200 ml of abs. toluene. After a further 10 minutes, the mixture is concentrated by evaporation *in vacuo* and the residue is partitioned between ethyl acetate/citrate buffer (pH 4). After separation by preparative HPLC, the evaporation residue

of the organic phase yields 15 mg of epothilon A.

75 litres of culture are grown as described in the basis patent and are used for inoculation in a production fermenter containing 700 litres of production medium consisting of 0.8% starch, 0.2% glucose, 0.2% soya flour, 0.2% yeast extract, 0.1% $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 0.1% $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 8mg/litre of Fe-EDTA, pH=7.4 and optionally 15 litres of Amberlite XAD-16 adsorber resin. Fermentation takes 7 to 10 days at 30°C, with aeration with 2 m³ air/h. The pO₂ is maintained at 30% by regulating the rotary speed.

After page 24, please insert the following:

Example 17



[1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6-ene-5,9-dione

A. [1S-[1R*,3R*(E),7R*,10S*,11S*,12R*,16S*]]-7,11-Hydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadecane-5,9-dione, 7,11-diformate.

Formic acid (0.95 ml, 25 mmol, 5.0 equiv), 4-N,N-dimethylaminopyridine (1.3 g, 11 mmol), and triethylamine (7.0 ml, 49 mmol) were added to a solution of epothilone B (2.5 g, 5.0 mmol) in CH_2Cl_2 (100 ml). The reaction mixture was cooled to -15° C. and acetic anhydride

(2.3 ml, 25 mmol) was added over 5 minutes. The reaction mixture was stirred for 15 minutes at -15° C. then warmed to room temperature and stirred for 30 minutes. The reaction mixture was quenched with pH 7.0 phosphate buffer, and the organic layer extracted with CH₂Cl₂ (3x100 ml). The combined organic extracts were washed with 1N HCl (1x100 ml) and 10% NaHCO₃ (1x100 ml). The organic layer was dried (Na₂SO₄), filtered, and concentrated under vacuum to give Compound A (2.8 g, 100%), a glassy solid, which was used without further purification.

MS (M+H)⁺564

TLC: R_f=0.71 (9/1 CH₂Cl₂ /acetone, visualization with UV)

B. [1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Formyloxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1.0]heptadec-6-ene-5,9-dione, 11-formate.

1,8-Diazabicyclo[5.4.0]undec-7-ene (7.3 ml, 49 mmol, 10 equiv), was added over 5 minutes to a solution of Compound A (2.8 g, 4.9 mmol) in CH₂Cl₂ (140 ml) at room temperature. The reaction mixture was stirred at room temperature for one hour. The reaction mixture was quenched with pH 4.0 phosphate buffer and extracted with CH₂Cl₂ (3x200 ml). The combined organic extracts were washed with 10% NaHCO₃ (1x200 ml). The organic layer was dried (Na₂SO₄), filtered, and concentrated under vacuum to afford Compound B (2.5 g, 100%), a glassy solid, which was used without further purification.

MS (M+H)⁺518

TLC: R_f=0.76 (9/1 CH₂Cl₂ /acetone, visualization with UV)

C. [1S-[1R*,3R*(E),10S*,11S*,12R*,16S*]]-11-Hydroxy-8,8,10,12,16-pentamethyl- 3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4,17-dioxabicyclo[14.1. 0]heptadec-6-ene-5,9-dione.

2M Ammonia in methanol (20 ml, 40 mmol) was added to a solution of Compound B (2.5 g, 4.8 mmol) in methanol (100 ml) at room temperature. The reaction mixture was stirred at room temperature for four hours. The reaction mixture was concentrated under vacuum. The residue was purified by flash chromatography (eluting with 19/1 CH₂Cl₂ /acetone) to afford the title compound (2.1 g, 89%), as a glassy white solid.

MS (M+H)⁺ 490

TLC: R_f=0.41 (9/1 CH₂ Cl₂ /acetone, visualization with UV)

Elemental analysis for C₂₇H₃₉NO₈S.0.22H₂O Calc: C, 65.70; H, 8.05; N, 2.84 Found: C, 65.69; H, 8.12; N, 2.77.